PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Brown *et al.*
Application No.	:	09/889,203
Filed	:	March 13, 2002
For	:	A COMPOSITION AND METHODS FOR THE ENHANCEMENT OF THE EFFICACY OF DRUGS
Examiner	:	B. M. Fubara
Art Unit	:	1615
Docket No.	:	650064.406USPC
Date	:	February 16, 2002

DECLARATION OF TRACEY BROWN, Ph.D.

Commissioner for Patents
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Washington, D.C. 20231

I, Dr Tracey Brown, do declare as follows:

1. I am an inventor of subject matter described and claimed in the above-captioned application (the "Application"). I reside at **23 Norwood St, Flemington, Victoria, Australia**, and I am a citizen of Australia. I am a pharmaceutical biochemist and ***attach*** my Curriculum Vitae as Exhibit A to evidence my standing in the field.

2. I have read and understand the application which currently claims methods of enhancing the efficacy of a cytotoxic or anti-neoplastic drug for a cancer cell, comprising administering hyaluronan (HA) and a cytotoxic or anti-neoplastic drug. My intellectual property advisors have explained that an Office Action has issued which *inter*

alia alleges that the invention lacks novelty and is obvious in light of Falk *et al.* (US Patent No. 5,985,850). My advisors have asked me to provide evidence of the efficacy of HA at particular molecular weights.

3. I have conducted numerous experiments that demonstrate that HA at a molecular weight of between 400 and 900 kDa increases the efficacy of a cytotoxic or anti-neoplastic drug against a cancer cell when compared to forms of HA having a lower molecular weight. The reasons for the differences between the different sized HA forms and the data demonstrating these differences are outlined below.

4. HA is a linear biopolymer of repeating units of glucuronic acid and N-acetyl glucosamine. As the monomers are linked in an alternating bond sequence, each monomer presents alternating hydrophobic and hydrophilic patches. This results in a "twisted ribbon" primary structure. As can be seen from Figures 1A and B in Exhibit B, low and high molecular weight HAs form different tertiary structures. As represented in Figure 1A, high molecular weight HA allows small molecules to be entrained within the structure. As the molecular weight of the HA increases, the complexity of the tertiary structure increases and the residence time of small molecules entrained therein increases. This is in contrast to the tertiary structure of an HA with a low molecular weight (i.e. an HA having a molecular weight below 400 kDa). Lower molecular weight forms of HAs are incapable of forming the complex tertiary structure required for effective small molecule entrainment. Accordingly, unlike the higher molecular weight forms of HA, low molecular weight HAs are unable to entrain the cytotoxic or anti-neoplastic drug and hence cannot deliver these drugs to the target site.

5. Falk *et al.* (US Patent No. 5,985,850) disclose formulations comprising HA with a lower molecular weight than contemplated by the present invention and these formulations function in a different manner. The formulations of Falk *et al.* use HA to exclusively enhance the penetration and transport of a drug *through* the tissue surrounding the various cellular elements of a tumor by altering the penetration characteristics of the surrounding tissue (see col. 2, lines 37-42 and 61-67; col. 10, lines 39-43; col. 11, lines 24-28 and 58-65; col. 12, lines 27-34; and col. 13, lines 1-4, 29-35

and 61-67). Furthermore, Falk *et al.* state that "[*preferably the hyaluronic acid is mixed with water and the fraction of hyaluronic acid fraction has a mean average molecular weight within the range of 150,000-225,000*". As discussed above, an HA with a molecular weight within this range would not function in the same manner as an HA having a molecular weight of greater than 400 kDa. Therefore, the formulations described by Falk *et al.* could not act in the same manner as the formulations of the Invention and hence the HA formulations *are* not the same and function differently. This highlights the significance of the choice of size of HA.

6. Such differences are further illustrated in a series of experiments in which water solutions of radiolabelled hyaluronan of different molecular weight were applied to the skin. As can be seen in Figure 2 (Exhibit C), HAs having a molecular weight of 250k daltons (low) were able to penetrate across the skin, however, HAs having a molecular weight of 700k daltons (high) were not. These data clearly demonstrate that HAs of different molecular weight function differently and represent distinct formulations.

7. On behalf of the Assignee, three phase I clinical trials and one phase II clinical trial have been conducted based on the use of HA with selected chemotherapeutic drugs.

These studies are:

phase I:	HA [890 kdalton] + 5-fluorouracil
phase I:	HA [890 kdalton] + doxorubicin
phase I:	HA [850 kdalton] + Irinotecan
phase II:	HA 825 kDa + Irinotecan for advanced colorectal cancer

The HA used in the phase II clinical trial had a polydispersity of 1.7. This means that the HA is a mixture of polymers of different molecular weights, with the mode (the molecular weight that occurs most frequently) centered at 825 kDa. The range of the modal distribution, the polydispersity, is defined as:

$$\frac{\text{(the weight average molecular weight of the sample)}}{\text{(the number average molecular weight of the sample)}}$$

In practical terms, a sample of 1.7 polydispersity has representative polymers from at least 700 kDa to at least 1,000 kDa.

The phase II clinical trial conducted on behalf of the Assignee was a controlled clinical trial in which 38 patients received irinotecan and 41 patients received the HA + Irinotecan combination. All patients had the same cancer type and had previously failed treatment.

The trial demonstrated that those patients receiving the HA + Irinotecan treatment had better disease control and treatment tolerance resulting in very significant indicators of efficacy.

Patients on Irinotecan alone had cancer which progressed in about 2.4 months. However, those patients who were treated with HA + Irinotecan did not progress to disease until about 5.2 months.

The progression free survival for patients receiving irinotecan was 8.0 months. This period was extended to 10.1 months for patients on the HA + Irinotecan arm.

These results have been accepted by the Federal Drug Administration (FDA) who has allowed direct entry into a phase III trial in the USA.

8. In addition, the use of an HA having a high molecular weight is counterintuitive due to the high viscosity of such compositions. Viscosity is a property of all liquids and is described as the resistance that a liquid exhibits to the flow of one layer over another. This is particularly relevant in biological and anatomical situations such as blood vessels, because the blood vessels are typically narrow. Small changes in viscosity lead to a more pronounced flow restriction in narrow vessels than in wider vessels.

The normal relative serum viscosity ranges from 1.4-1.8 units (reported as Centipoise). As the concentration of serum proteins, or other biomolecules increase, the blood becomes more viscous. When the viscosity increases to about 4 centipoise, the serum is considered to be hyperviscous. A serum viscosity of between 4 and 5 centipoise is usually sub-clinical hyperviscosity syndrome, while people with serum viscosities above 5 centipoise exhibit clinical symptoms of hyperviscosity syndrome. Clinical symptoms of this syndrome include mucosal bleeding, visual changes, and neurologic symptoms. Other manifestations may include heart failure, shortness of breath, hypoxia, fatigue, and anorexia. Constitutional symptoms and cardio-respiratory symptoms also contribute to the clinical presentation.

Viscosity increases in serum from HA are directly related to the molecular weight of the HA and the concentration of the HA. The following table demonstrates the relationship between viscosity and molecular weight for HA solutions. [Please refer to: *Ogston, A.G., & Steiner, J.E., J. Physiol., 1953, 119, 244-52][Soltes et. al., Carb. Research., 2007, 342, 1071-1077*].

Molecular weight (kDa)	Viscosity @ 2.5 mg / ml
90	1.50
426	4.20
659	5.94
804	8.45
809	9.78
1017	6.33
1292	8.33
1340	11.00
1378	11.13
1553	11.75

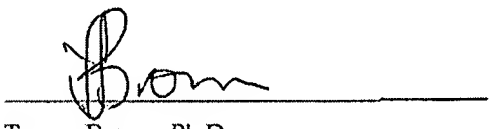
Clearly, as the molecular weight of the HA increases for a given concentration of HA, the viscosity increases proportionally, such that at about 400 kDa, the solutions fall within the range of sub-clinical hyperviscosity syndrome (exceeds 4 centipoise) and at about 600

kDa the solutions fall within the range of symptomatic hyperviscosity syndrome (exceeds 5 centipoise).

Quite apart from hyperviscosity syndrome which is to be avoided, the intravenous injection of highly viscous materials and the deliberate inducement of even moderate elevations in serum viscosity is potentially dangerous and may result in the formation of occlusions to blood vessels, or the formation of emboli or thromboses. As such it is counter-intuitive to develop treatments which deliberately or consequentially raise serum viscosity.

In the phase II clinical trials which have been conducted on behalf of the Assignee, a formulation comprising a solution of HA of between 2 mg/mL and 4 mg/mL mixed with a cytotoxic agent is provided to patients in need. These solutions have a high initial viscosity and elevate serum viscosity as a result. No adverse side effects was observed in patients receiving our claimed therapeutic compositions.

9. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false statements, and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.



Tracey Brown, Ph.D.

17 July 2008

Date

Figure 1A

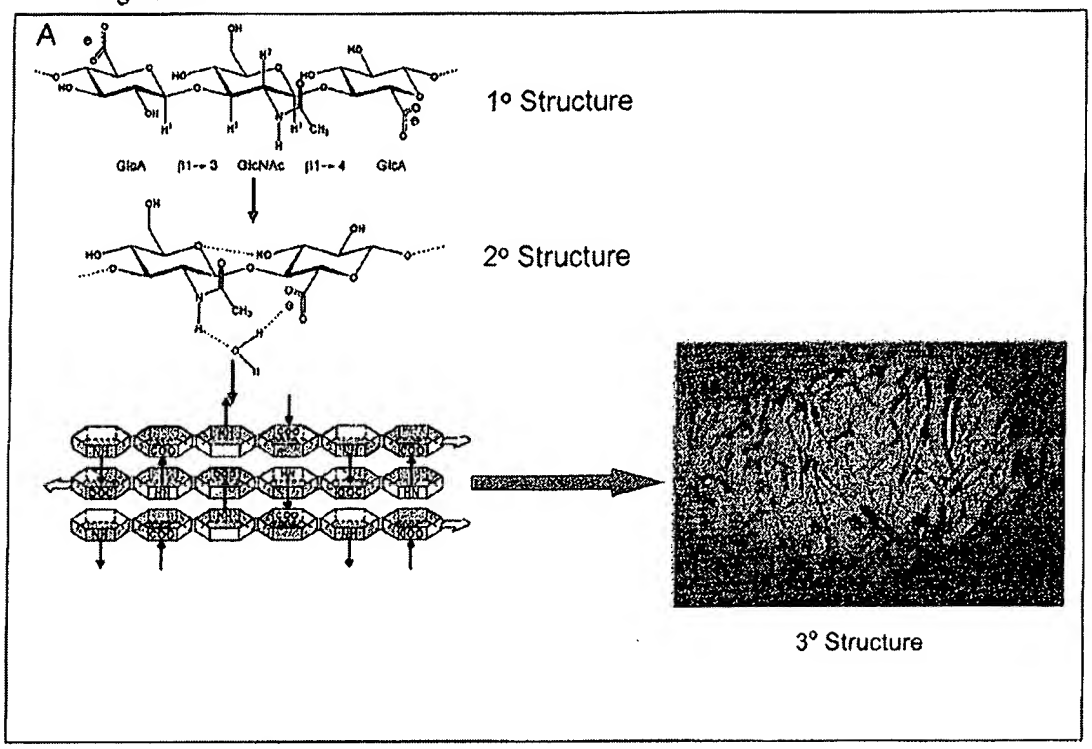


Figure 1B

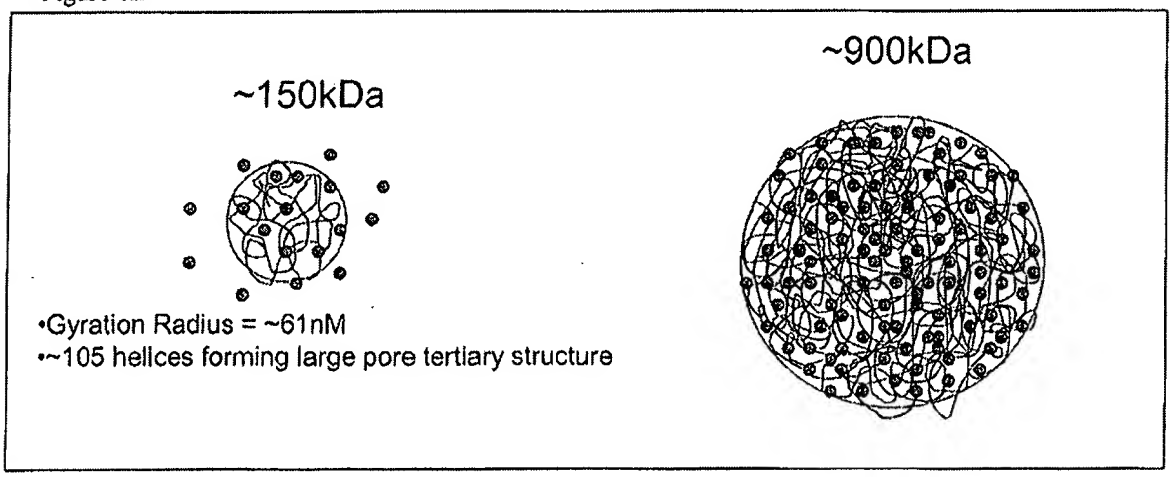
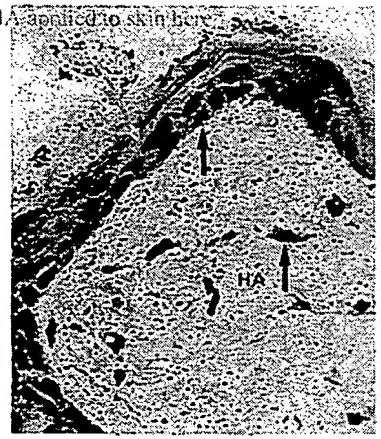
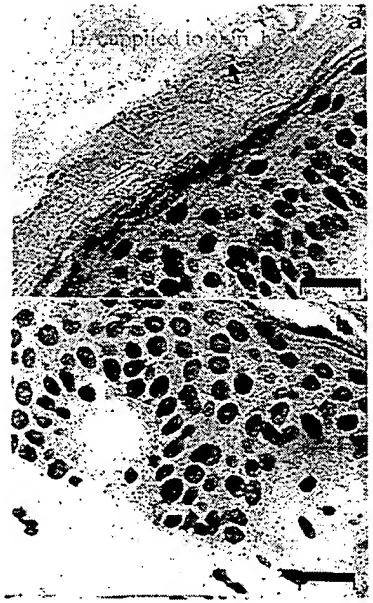


Figure 2A



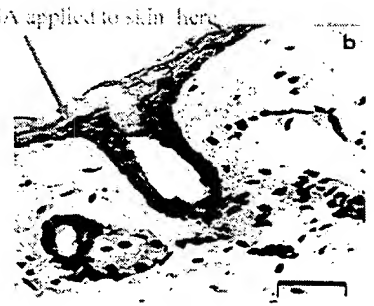
Absorption of topically applied
250kDa HA

Figure 2B



Absorption of topically applied
400kDa HA

Figure 2C



Absorption of topically applied
700kDa HA

Figure 2:
The red arrows indicate where radiolabelled hyaluronan was topically applied. The skin sections were autoradiographically processed which resulted in any radiolabelled hyaluronan presenting as tiny black dots (larger black dots are cells). As seen in Figure 2A, the majority of applied 250kDa HA was able to enter the skin and lodge in the epidermis and dermal layers. In comparison, in Figure 2B, very few black dots (HA) are evident beyond the epidermis of the skin demonstrating that the 400kDa HA had limited ability to penetrate the skin due to its large volume. Figure 2C clearly lacks black dots (HA) on the surface of the skin or in the skin which clearly demonstrates that 700kDa HA is too large to penetrate skin and it merely flakes from the surface of the skin instead of percutaneously entering into the stratum corneum of the skin.